Prenatal Se concentrations and anthropometry at birth in the INMA study

Prenatal Se concentrations and anthropometry at birth in the INMA study (Spain)

Manuel Lozano[1,8], Mario Murcia[2,3], Raquel Soler-Blasco[2], Carmen Iñiguez[3,9], Amaia Irizar[5], Aitana Lertxundi[3,4,5], Mikel Basterrechea[3,4,5], Loreto Santa Marina[3,4,5], Rubén Amorós[6], Karin Broberg[7], Ferran Ballester[2,3,10], Sabrina Llop*[2,3]

[1] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health

[2] Epidemiology and Environmental Health Joint Research Unit, FISABIO–Universitat Jaume I–Universitat de València, Valencia, Spain

[3] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Madrid, Spain

[4] Preventive Medicine and Public Health Department. University of Basque Country, UPV/EHU, Leioa, Bizkaia, Spain

[5] Health Research Institute, BIODONOSTIA, San Sebastian, Gipuzkoa, Spain.

[6] School of Mathematics, Peter Guthrie Tait Road, University of Edinburgh, EH9 3FD Edinburgh, United Kingdom

[7] Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

[8] Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine Department. Universitat de València, València, Spain

[9] Department of Statistics and Computational Research. Universitat de València, València, Spain

[10] Nursing Department, Universitat de València, València, Spain

Corresponding author

*Sabrina Llop. Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (FISABIO), Av. Catalunya 21, 46020 Valencia, Spain. E-mail address: llop_sab@gva.es

Abstract

We assessed whether prenatal selenium (Se) exposure is associated with anthropometry at birth, placental weight and gestational age. Study subjects were 1249 mother-child pairs from the Valencia and Gipuzkoa cohorts of the Spanish Childhood and Environment Project (INMA, 2003–2008). Se was determined in serum samples taken at the first trimester of pregnancy. Socio-demographic and dietary characteristics were also collected by questionnaires. Mean (SD) serum Se concentration was 79.57 (9.64) μg/L. Se showed weak associations with both head circumference and gestational age. The association between serum Se concentration and birth weight and length was negative, and direct for placental weight and probability of preterm birth, although the coefficients did not reach statistical significance. Individuals with total mercury (THg) levels >15 μg/L reversed the serum Se concentration effect on head circumference. Significant interactions were found between sex and both gestational age and prematurity. Spontaneous birth gestational ages were estimated to be lower for males and their probability of prematurity was higher. In conclusion, prenatal Se exposure may be associated with lower head circumference and lower gestational ages at spontaneous birth. Interactions with THg exposure and gender should be considered when assessing these relationships.

Keywords

Selenium, prenatal exposure, birth anthropometry, gestational age, preterm birth

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1 Introduction

Selenium (Se) is an essential trace mineral of fundamental importance to human health, since it is involved in antioxidant processes and has therapeutic aspects due to its chemo-preventive, anti-inflammatory and antiviral properties (Rayman, 2012).

Se is present in the soil and enters the food chain via plants in its inorganic form (Peters et al., 2016). On a global scale, Se availability in the soil varies between areas. Low Se content is observed in volcanic regions and in regions with an acidic soil. Consequently, Se deficiency-linked disorders in humans (such as gastrointestinal and prostate cancer, cardiovascular disease, diabetes, or compromised fertility) have been documented in such areas, and more so if the food is produced mainly locally (Papp et al., 2007). In plants, Se is converted into organic forms such as selenomethionine (SeMet) and selenocisteine (Sec). SeMet is the major selenocompound in cereal grains and legumes, and it serves as a major precursor for Sec synthesis in animals (Peters et al., 2016). Sec is the most active biological form of Se and participates in the synthesis of selenoproteins, such as glutathione peroxidase (GPx), selenoprotein P (SelP) and thioredoxin reductase (Trx), which play an important role in preventing damage from oxidative stress (Whanger, 2002).

During a normal pregnancy, maternal oxidative stress rises and there is a systemic inflammatory response, largely triggered by the invasion of the placenta into maternal uterine tissue (Hung et al., 2010). The ability of selenoproteins to reduce oxidative stress, endoplasmic-reticulum stress and inflammation, and to protect the endothelium, control eicosanoid production, regulate vascular tone and reduce infection, is likely to be important for the health and development of the fetus (Rayman, 2016). However, maternal whole blood Se concentration falls substantially with increasing gestational age (e.g. by 12% between the first and third trimester) because of both plasma volume expansion and Se transfer to the fetus (Rayman et al., 2014).

Previous research assessing the effect of Se concentrations on anthropometric birth outcomes is scarce and inconclusive. Thus, two studies have evaluated the association between decreasing maternal Se concentrations and the higher probability of preterm birth in 113 woman-and-child pairs from Nigeria (Okunade et al., 2018) and 1197 from Holland (Rayman et al., 2011), with statistically significant results. However, regarding the anthropometric birth outcomes such as low birth weight, birth length and head circumference the results were more heterogeneous. This inconsistency in results could be related to reduced sample size. Thus, maternal Se was positively associated with anthropometric birth outcomes in women from UK (n=233), China (n=408), Saudi Arabia (n=249) and USA (n=271) (Al-Saleh et al., 2014; Bogden et al., 2006; Sun et al., 2018; Wells et al., 2016a). Other studies with lower sample sizes have not shown any statistical relationship, such as a study carried out in 81 woman-and-child pairs from China (Hu et al., 2015) and another one conducted in 40 Chilean woman-and-child pairs (Llanos and Ronco, 2009).

Birth anthropometric measures predict adverse pregnancy outcomes that might be associated with a high risk of mortality, morbidity and disability during infancy and childhood as well other chronic diseases later in life (Zheng et al., 2016). For example, fetal growth retardation has been associated with a relatively greater probability of coronary heart disease, hypertension and type 2 diabetes mellitus (Barker et al., 2005; Bhargava et al., 2004). Moreover, low birth weight has been related to cardiovascular disease (Osmond et al., 1993). Finally, prematurity has been associated with insulin resistance and glucose intolerance in pre-pubertal children (Hofman et al., 2004) that may track during young adulthood and which can be accompanied by elevated blood pressure (Hovi et al., 2007). Several studies have also found positive associations between birth weight and a higher probability of breast cancer (Dos Santos Silva et al., 2004). On the other hand, head circumference at birth and early childhood has been positively associated with a range of neuropsychological outcomes, including protective associations with ADHD symptoms (Ferrer et al., 2019).

Recommended Se intake through diet is set at 70 µg/day and 55 µg/day for men and women, respectively, plus a further 10–20 µg/day during pregnancy and lactation (James, 2000; National Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989). The most conservative reference of Se in plasma is 70 µg/L, which is considered the minimum value to reach maximum GPx activity (López-Bellido Garrido and López Bellido, 2013). In Spain, the mean Se intake range is 21–75 µg/day and serum mean ± standard deviation (SD) is 80.7 ± 10 µg/L (Rivas et al., 2012), which is close to the recommendations. Most dietary Se comes from bread, cereal, meat, fish and poultry and, increasingly in recent times, dietary supplements (Alexander, 2015). In Spain, the Mediterranean dietary pattern highlights fish consumption (Sofi et al., 2013; Willett et al., 1995), which is a substantial source of Se and Hg. Se content in fish varies from about 12 µg/100 g in lean fish to over 70 µg/100 g in canned tuna (Hatfield et al., 2016). However, the bioavailability of Se (14% of Se content in ingested food) may be modified by the presence of heavy metals, such as mercury (Hg), which may decrease Se absorption via chelation and precipitation (Peters et al., 2016; Wells et al., 2016a). THg is also able to affect Se transport across membranes and tissue distribution by binding to inorganic Se and forming an insoluble, stable and inert Hg:Se complex, as well as binding to the Se site in selenoproteins and permanently inhibiting their function, thereby disrupting the intracellular redox environment (Spiller, 2018).

The *INMA – INfancia y Medio Ambiente* (Environment and Childhood) Project (http://www.proyectoinma.org/) is a network of seven birth cohorts in Spain (Valencia, Granada, Menorca, Sabadell, Ribera d'Ebre, Gipuzkoa and Asturias) that aims to study the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development (Guxens et al., 2012). The objective of the present study was to evaluate the association between maternal Se serum levels and anthropometric birth outcomes in the INMA-Valencia and INMA-Gipuzkoa cohorts.

2 Materials and Methods

2.1 Study population

Study subjects were participants in those two cohorts of the INMA Project with available serum Se data. Between 2003 and 2008, 1493 pregnant women were recruited at the beginning of their pregnancy in the geographical areas of Valencia and Gipuzkoa, Spain. The inclusion criteria were: at least 16 years of age, 10–13 weeks of gestation, singleton pregnancy, intention of undergoing follow-up and delivery in the corresponding center of reference, non-assisted conception and no impediment for communication. A final sample of 1249 mother-child pairs (83.7%, Valencia n=655, Gipuzkoa n=594), for whom serum Se concentrations and new-born anthropometric data were available, completed the study until delivery. The Hospital Ethics Committees of each area approved these research protocols and informed consent was obtained from all participants.

2.2 Se and THg concentrations

Se concentrations were determined in serum samples taken at the end of the first trimester of pregnancy (mean (SD) = 13.1 (1.2) weeks of gestation). After separation of serum by centrifugation, samples were stored at –80^{o}C and transported frozen to the Karolinska Institutet, Sweden, for analysis. The serum Se concentrations were determined by inductively coupled plasma mass spectrometry ((ICPMS); Agilent 7700x, Agilent Technologies, Tokyo, Japan) with the collision/reaction cell system in hydrogen mode. Approximately 120 µg of serum was diluted 1:25 in an alkaline solution containing 1% ammonium hydroxide (NH_4OH) (Merck, Darmstadt, Germany), 2% w/w 1-butanol, 0.05% ethylenediaminetetraacetic acid (EDTA), 0.05% Triton X-100 and 20 ng/g of internal standards (Sc-45, Ge-72, Rh-103; CPI International, Amsterdam, Netherlands). Samples were then sonicated and centrifuged for 5 min each. Analytical quality control was performed by inclusion of reference materials (Seronorm™:Trace Elements serum lot MI0181, Trace Elements whole blood L-1 lot 1406263 and L-2 lot 1406264, and Medisafe

serum L-2 lot 28342). The values obtained were within the analytical range or within 20% of the analytical value for all reference materials (Amorós et al., 2018a). The limit of detection was 0.03 µg/L and no samples had concentrations below this value serum Se concentrations were corrected according to the variations in three daily measures of the Seronorm™ (lot MI0181) reference material. The correction was performed by adding to each measure the difference between the daily mean of the reference measures and the overall mean of the reference measures (Amorós et al., 2018a, 2018b). Cord blood total mercury (THg) concentrations were determined as explained elsewhere (Murcia et al., 2016).

2.3 Birth anthropometric measures

Gestational age was defined on the basis of the self-reported last menstrual period. An early crown-rump length measurement was also available and was used for gestational dating when the difference with the last menstrual period was ≥ 7 days (12% of the cases). Women for whom this difference exceeded 3 weeks (0.7%) were removed from the study to avoid possible bias. Preterm births were those with a gestational age below 37 weeks. Type of delivery was categorized as spontaneous, induced or caesarean, while births were divided into preterm versus full-term.

Birth weight was measured by the midwife attending the birth, whereas birth length and head circumference (HC) were measured by a nurse when the new-born arrived at the hospital ward within the first 12 hours of life. Placental weight was recorded by the midwife after birth. Growth curves for anthropometric measures and placental weight were fitted to further standardize them to week 40 of gestation using the Box-Cox power exponential method (Rigby and Stasinopoulos, 2004) adjusting by cohort and sex.

2.4 Covariates and potential confounders

The recruited women completed two questionnaires during their pregnancy, one at the first trimester (mean (SD) = 13.2 (1.6) weeks of gestation) and the other at the third trimester (mean (SD) = 32.6 (2.3) weeks of gestation). The questionnaires were administered by trained interviewers and focused on dietary, socio-demographic, environmental and lifestyle information during pregnancy. The maternal covariates and potential confounders collected were selected based on previous INMA studies on Se (Amorós et al., 2018a, 2018b): country of birth (Spain, other), age (quantitative and categorized as age <25, 25–29, 30–34, ≥ 35 years), weight, height, body mass index before pregnancy, level of education (up to primary studies, secondary studies, university), parity (0, 1, ≥ 2), type of delivery (spontaneous, induced and caesarean), area of residence (urban, semi-urban, rural), employment during pregnancy (non-worker, worker), smoker at the first trimester (no, yes), overall exposure to smoking (number of exposure environments such as home, workplace, restaurant services and leisure areas), season of blood sampling, and the newborn's sex. We also obtained data on paternal age, height, body mass index, employment and level of education. Parental social class was defined from the maternal or paternal occupation during pregnancy with the highest social class, according to a widely used Spanish adaptation of the International Standard Classification of Occupations, approved in 1988 (ISCO88) (Class I + II: managerial jobs, senior technical staff and commercial managers; class III: skilled non-manual workers; and class IV + V: manual and unskilled workers).

Information on diet during pregnancy was collected in order to assess potential dietetic sources of serum Se concentrations by using a semi-quantitative food frequency questionnaire (FFQ) in the first trimester. This FFQ was validated in this population of pregnant women, with good reproducibility being observed for nutrient and food intake (Vioque et al., 2013).

We obtained data (expressed in grams and servings per week) on the intake of seafood, meat, cereals and pasta, legumes, nuts, fruits, vegetables, eggs, dairy products, potatoes and bread.

Due to the large range of both Se and THg concentrations depending on the type of fish, seafood was included as four different categories based on the regular consumption of the Spanish population: lean fish, oily fish, canned tuna and processed lean fish. Self-reported maternal consumption of multivitamin supplements was obtained from an additional questionnaire at the first trimester of pregnancy. Se supplementation was also categorized into two groups (Se supplementation vs. no Se supplementation).

We categorized this variable (< 15 vs. ≥ 15 µg/L) according to the equivalent for the WHO Provisional Tolerable Weekly Intake (1.6 µg/kg of body weight per week) ("Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food," 2012).

2.5 Statistical Analysis

We analyzed the association between serum Se concentrations and anthropometric measures using multivariable generalized linear regressions models (GLM): linear regression models for continuous Gaussian outcomes (birth weight, birth length, head circumference, and placental weight) and logistic regression models for the binary outcome (prematurity). Cox regression (proportional hazards model) for the length of gestation was also used due to the lack of normality of the outcome and the presence of censoring. Spontaneous birth was therefore defined as the event in Cox regression, while elective delivery was considered to be censoring (Murcia et al., 2016). Survival curves were built for descriptive purposes, with maternal serum Se concentrations categorized as < 70 or ≥70 µg/L (James, 2000).

We adjusted confounders and predictors of the outcomes in accordance with the following procedure: (1) Basal models were built following a backward elimination procedure considering all the 1249 mother-child pairs: starting from models including all covariates related to each response at $p<0.20$ in univariate analysis, we sequentially excluded those variables with an adjusted $p>0.10$. Cohort was included in all models regardless of their statistical significance. (2)

Serum Se concentration was then incorporated and possible confounders were subsequently included if they changed the magnitude of the main effect in a significant way with a 5% significance level (Lee, 2014). In cases in which they were not included in the basal models, maternal age, country of birth, maternal education, season of blood sampling, and seafood consumption were assessed as potential confounders on the basis of previous knowledge regarding their potential relation with maternal Se status (Al-Saleh et al., 2014; Amorós et al., 2018a). Energy-adjusted intakes were computed using the residual method, where the residuals were calculated from a linear regression, with the natural logarithm of the food group modeled as the dependent variable and the natural logarithm of total energy intake as the independent variable.

Generalised additive models (GAM) using natural cubic smoothing splines (one or two internal knots) were employed to assess the linearity of the relationship between each outcome and serum Se concentrations by graphical observation and the Akaike information criterion (AIC). Segmented models were used to determine possible break-points in the linear association between each outcome and serum Se concentrations, and the Davis test was also used to find the potential location of these break-points (Muggeo, 2003).

Effect modifications (interaction) by child sex, categorised cord blood THg concentrations and season of sampling were assessed. To do so, the interaction effect of these variables with serum Se concentration was tested using AIC scores between the GLM and GAM models with and without interaction.

Finally, sensitivity analyses were performed to assess changes in serum Se concentrations by building a multivariate model only using participants without Se supplementation and excluding preterm births. Analyses were conducted with the statistical software R, version 3.4.3 (R core Team, 2017).

3 Results

Descriptive statistics of the study variables are displayed in Table 1. Mean age of mothers included in the study at the time of conception was 30.7 years, 91.8% were born in Spain, 85.6% were working at the first trimester of pregnancy, 54.4% were primiparous, 18.7% smoked at sampling and 65.9% were exposed to sources of smoke, and 7.5% lived in rural areas. The mean birth weight was 3,343 g and the mean gestational length was 39.7 weeks, with 4.2% preterm births.

The mean (SD) maternal serum Se concentration was 79.57 (9.64) µg/L. Lower serum Se levels were found in Gipuzkoa (77.08 (10.62) µg/L) than in Valencia (81.93 (7.92) µg/L). Statistically significant differences were found between the two cohorts (t-test p-value <0.001). The geometric mean (SD) THg in cord blood was 8.45 (2.17) µg/L.

Table 2 shows correlations between serum Se concentrations, THg and the four seafood intake variables, which are likely to contain Hg, calculated considering the whole sample and adjusted for cohort. The table also shows that the correlation between Se and Hg also remains significant after mutual adjustment for Se, Hg, and seafood intake. The correlation between serum Se concentrations and seafood intake was significant in the Gipuzkoa cohort.

The variables included in the multivariate models for each anthropometric outcome can be observed in Figure 1. Multivariate GLMs showed a fit improvement (lower AIC score) compared to the GAM for the association between maternal serum Se concentrations and each outcome. Direct observation of the estimated splines of the GAMs did not show any non-linear associations, and the Davis test did not find any break-point with statistical significance.

The serum Se concentrations effect was marginally statistically significant in both head circumference (β = –0.007; p-value = 0.08) and gestational age (HR 1.007; p-value = 0.06) (Table 3). The positive relation between serum Se concentrations and gestational age should be

considered as an increased risk of spontaneous delivery (event of survival analysis). The association between maternal serum Se concentrations and other anthropometric outcomes was negative for birth weight and length and direct for probability of preterm birth and placental weight, although the coefficients did not reach statistical significance.

Table 3 also shows serum Se concentrations effect modifications (interactions) by categorised cord blood THg concentrations, sex of the child and season of sampling. Individuals with THg levels > 15 µg/L reversed the serum Se concentrations effect on head circumference with marginal significance (Figure 2). A slight improvement in terms of AIC was found on adding the interaction term to the GLM in the model (AIC 3783.90 and 3784.07 with and without interaction term, respectively). Child's sex also showed a significant serum Se concentrations effect modification on both gestational age (risk of spontaneous birth) and prematurity (Supplemental Figure S3 and S4, respectively). Females had a lower risk of spontaneous birth (β interaction term –0.017, p-value = 0.02) than males, the AIC improved after adding the interaction term in the model (AIC 10783.57 and 10786.95 with and without interaction term, respectively). Direct observation of the survival curves for both sexes showed significant differences only for females whose mother's serum Se concentration was < 70 µg/L (p-value of log-rank test = 0.0074). Female sex had a lower probability of prematurity (β interaction term –0.068, p-value = 0.02), the AIC improved after adding the interaction term in the model (AIC 421.76 and 424.18 with and without interaction term, respectively). No seasonal interactions were found.

Sensitivity analyses were also carried out. Figure 2 shows adjusted associations between maternal serum Se concentrations and birth anthropometric measures: (1) raw models considering only exposure; (2) models adjusted by covariates as described in Figure 1; (3) models adjusted for covariates and confounders, as shown in Table 3; (4) models adjusted for covariates and confounders including interaction terms for sex, sampling season and THg, the

numeric results of which are also shown in Table 3; (5) models adjusted for covariates and confounders excluding 142 mothers who consumed dietary supplements that contained Se, which did not affect the estimates meaningfully, although the slight loss of serum Se concentrations significance in the head circumference model might be related to the chemical form of Se in dietary supplements; (6) models adjusted for covariates and confounders excluding 769 preterm births, which did not affect the estimates either; and (7) models adjusted for covariates and confounders with THg as an additional confounder in order to assess changes in Se coefficients, which also showed similar estimates. As can be seen in Supplemental Table S1, THg did not confound the relationship between serum Se concentrations and the outcomes. When models were additionally adjusted for THg, an apparent slight and non-significant change in the serum Se coefficients was observed. However, these changes are due exclusively to the reduction in the sample size and the consequent loss of statistical power. All models were adjusted for cohort.

4 Discussion

In this birth cohort study, we observed a weak inverse association between maternal serum Se concentrations in serum and child's head circumference at birth. Additionally, serum Se concentrations showed a marginally significant direct relation to a higher risk of spontaneous birth at lower gestational ages. The relation between maternal serum Se concentrations and other anthropometric outcomes was negative for birth weight and length, and direct for probability of preterm birth and placental weight, although the coefficients did not reach statistical significance. Maternal serum Se concentrations were 79.57 (9.64) µg/L at the first trimester of gestation, which are close to the recommendations set at 70 µg/L during pregnancy.

Previous research concerning the effect of Se on anthropometric birth outcomes is scarce and inconclusive. These studies have mainly evaluated the association between Se concentrations

and child birth weight, showing a protective effect of Se but showing some inconsistencies in results which may be due to different sources of dietary intake, sample sizes, matrices used to collect Se concentrations or residual confounding. For example, positive associations were found between maternal Se concentrations and birth weight in United Kingdom (mean (SD): 106.24 (2.99) µg/L in serum at the first trimester, n=233) (Bogden et al., 2006), as well as in China (geometric mean (95% confidence intervals (CI)): 156.58 (145.82–167.34) µg/L in maternal blood at the third trimester, n=209) (Sun et al., 2014). Considering cord blood, positive associations were also found with birth weight in Saudi Arabia (67.618 (12.897) µg/L, n=249) (Al-Saleh et al., 2014) and in USA (70.0 (68.5–71.4) µg/L, n=271) (Wells et al., 2016b). However, other studies with lower sample sizes have not shown any statistical relationship, such as a study carried out in 81 woman-and-child pairs from China in both maternal and cord blood (Hu et al., 2015) and another one conducted in 40 Chilean woman-and-child pairs by using placenta samples (Llanos and Ronco, 2009).The relationship between Se and other anthropometric outcomes such as length, head circumference, placental weight, as well as gestational age and prematurity has been barely studied to date. A weak but significant positive association between Se and gestational age (β = 0.31 days, 95% CI = 0.18–0.45) was also highlighted in the study carried out in USA mentioned above (Wells et al., 2016b). Furthermore, a positive association between cord blood Se and child's head circumference and placental weight was also observed in the Saudi study (Al-Saleh et al., 2014). However, an inverse relation between Se concentrations in placenta and placental weight was found in a small sample (n = 20) of Polish women (Zadrozna et al., 2009).

Contrary results to ours regarding prematurity were observed in two studies conducted in Holland (n=1167) and Nigeria (n=113). Women with Se concentrations below 72.6 µg/L in Holland (Rayman, 2016) and 70 µg/L in Nigeria (Okunade et al., 2018) had a higher probability of giving birth prematurely. The discrepancies between those results and present data could be explained by a non-linear relationship, a possibility that was not explored in any of the above

mentioned studies. However, the AIC analyses performed in our study indicated that the relationships between Se and anthropometric outcome were linear.

In the present study we have also observed a differentiated relationship between maternal serum Se levels and the newborn's head circumference at birth according to the level of THg in cord blood. The association between serum Se concentrations and the newborn's head circumference was negative for those with cord blood THg below 15 µg/L and positive for those with THg levels above 15 µg/L. The interaction p-value was marginally statistically significant. The Saudi study mentioned above also indicated marginally significant Hg–Se antagonistic interactions in placental samples (p = 0.091) that may have moderated the toxic effect of Hg on head circumference. However, other studies have also assessed interactions between the two metals without finding any significant effects. For example, in a study carried out in 15,444 Japanese women, which specifically assessed the relationship between maternal Se and THg concentrations and birth outcomes, a reduction in birth head circumference by 0.073 cm (95% CI: −0.134, −0.011) was observed in mothers with the highest THg levels, but without Se interaction (Kobayashi et al., 2019). Similarly, the study conducted in 271 children from USA mentioned earlier showed that interaction between Se and methylmercury had no significant effect on birth outcomes (Wells et al., 2016b). A recent review proposed that Hg interacts with Se by binding with both the inorganic Se form and Se sites in selenoproteins, which might explain why Hg causes oxidative stress (Spiller, 2018). According to this, results shown in this study regarding the inverse relationship between serum Se concentrations and children’s head circumference would be supported.

We have also observed a differentiated association between maternal serum Se levels and outcomes according to the child’s sex, since a higher probability of spontaneous birth and prematurity was seen in males. Possible explanations of this sex effect are greater body weight, increased susceptibility to complications of pregnancy, sex-linked biochemical processes, and

earlier conception in the fertile cycle (Zeitlin et al., 2002). The lower probability of prematurity observed for females in the present study has also been found in several studies. Among the most recent, a Turkish study carried out in 29,528 pregnant women, 7,382 of whom had preterm deliveries, showed a significantly lower prematurity in 24.3% of female versus 25.7% of male newborns (p-value = 0.004) (Kanmaz et al., 2019). However, no significant association between sex and preterm births was found in a sample of 2,505 pregnant women from UK, 230 of whom had spontaneous deliveries < 37 weeks (males n = 108 (8.7%), females n = 122 (9.6%); Relative Risk (95% CI) = 1.10 (0.86–1.41), p-value = 0.45) (Teoh et al., 2018).

Furthermore, our sensitivity analyses excluding participants who consumed dietary supplements and preterm births obtained similar estimates. However, these analyses resulted in the loss of serum Se significance regarding both the newborn's head circumference and a higher probability of spontaneous birth (versus caesarean or induced among non-preterm births) because of the decrease in the sample. Results with respect to head circumference were also observed in the Saudi study mentioned previously (Al-Saleh et al., 2014), since after the exclusion of preterm births Se significance disappeared. Authors suggest that Se may play a noteworthy role in the etiology of preterm births. Nevertheless, the US study (Wells et al., 2016b) which observed an association between Se and gestational age did not assess sensitivity analyses excluding preterm births and no other research about this association has been found.

There are some limitations in the present study: (1) there was a lack of Se speciation data. Associations observed in this study could be related to one or several of the Se forms present in the body and this fact could in turn be related to the specific form used in supplements, which have shown a specific compromise of Se significance in sensitivity analyses; (2) although we used a validated FFQ administered at the same time as blood sampling, some degree of misclassification in the maternal food intake is likely to be expected; and (3) the number of children with cord blood THg data available was lower than with maternal serum Se

concentrations data and this could affect the proper interpretation of statistical models with THg. This reduction affects the power of these models.

The main strengths of this study are: (1) the large size of the whole sample built with two distanced cohorts with differences in social class and education (higher in the Gipuzkoa cohort) and dietary pattern. Regional differences in the consumption of big oily fish species (higher in Valencia) could explain, in part, the different mean concentrations between cohorts. In our FFQ this category included fresh tuna and swordfish, the fish species with higher Se content (Olmedo et al., 2013) (2) the novelty of the statistical approach, which assesses both linear and non-linear associations between serum Se concentrations and anthropometric measures, and also the use of time-to-event analysis to control for the presence of censoring (induced births) in the analysis of the length of gestation, while also avoiding an inaccurate assumption of normality. The statistical analyses also include the performance of sensitivity analysis, confounding assessment and interaction analysis; and (3) the study's prospective design, which made it possible to obtain detailed information concerning maternal and child socio-demographic, dietary and life-style characteristics that may affect anthropometric outcomes.

5 Conclusion

We observed a weak linear association between maternal serum Se concentrations and lower circumference of the child's head at birth and higher risk of spontaneous birth (lower gestational age at birth), despite the fact that serum Se concentrations were within the range considered to be appropriate to ensure antioxidant capability. The non-significant tendencies observed with birth weight and length, as well as prematurity, show consistency regarding a detrimental effect of Se. Further studies are necessary in order to confirm these results.

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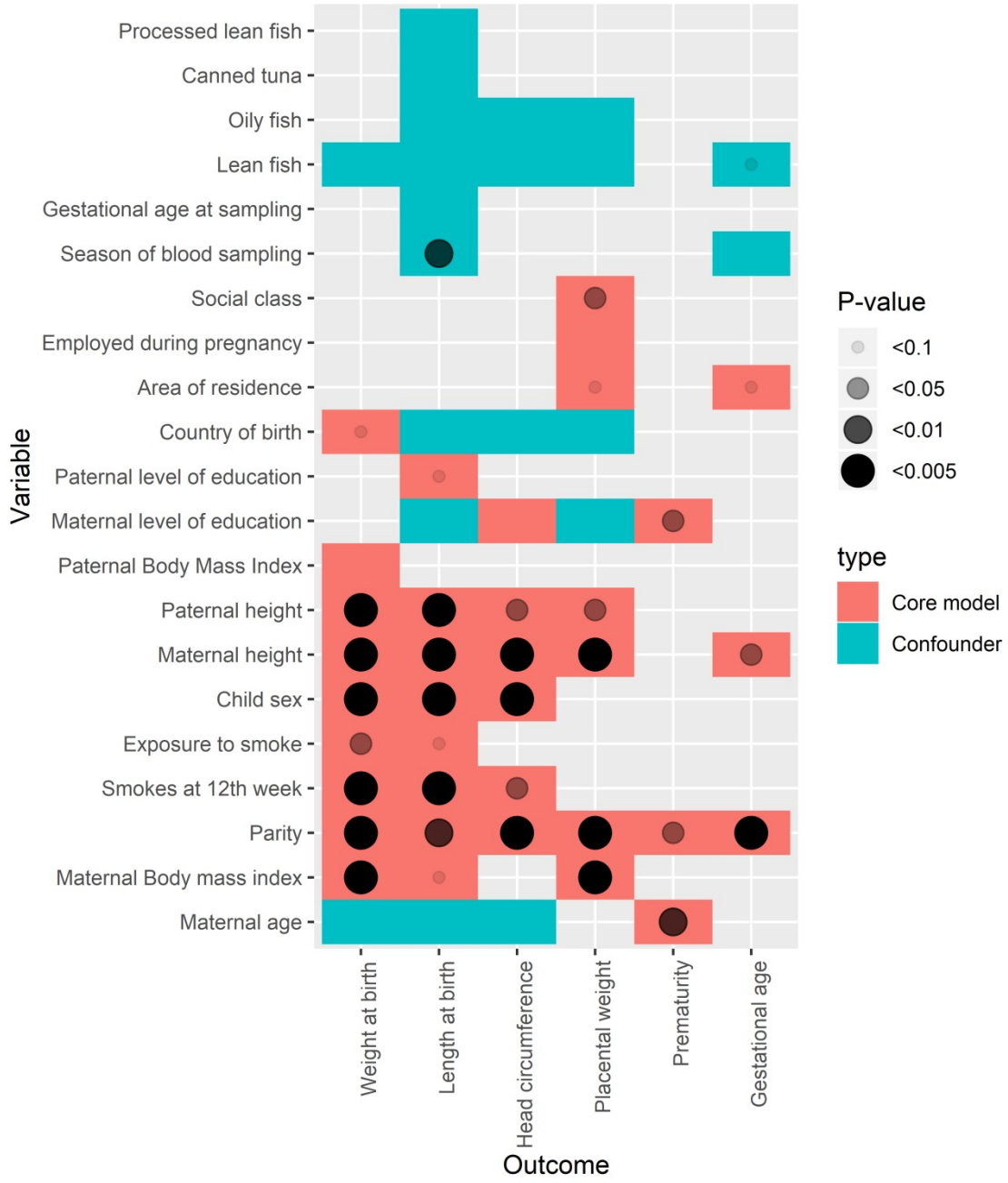
593**Figure 1.** Variables included in each of the multivariate models for the anthropometric outcomes and
594associated p-value range.



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598**Figure 2.** GLM for the association between maternal Se concentrations (measured at the first trimester
599of gestation) and child's head circumference at birth. INMA Project (2003–2008).

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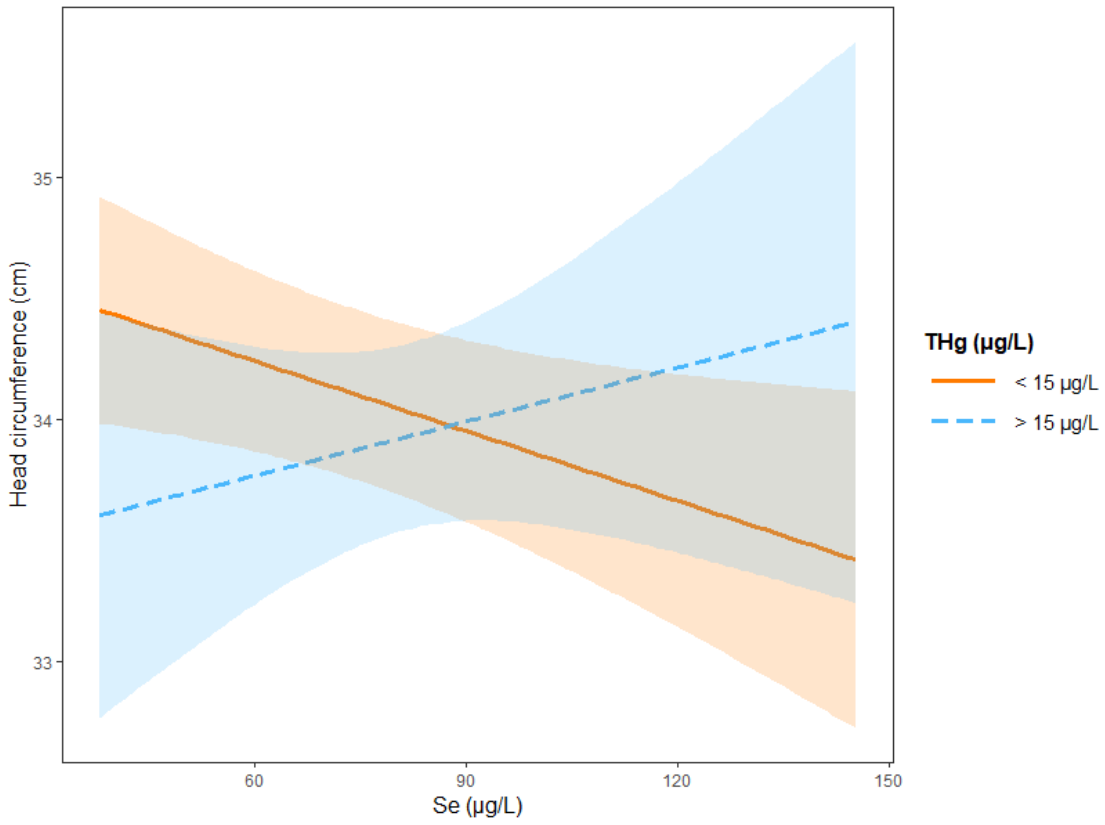

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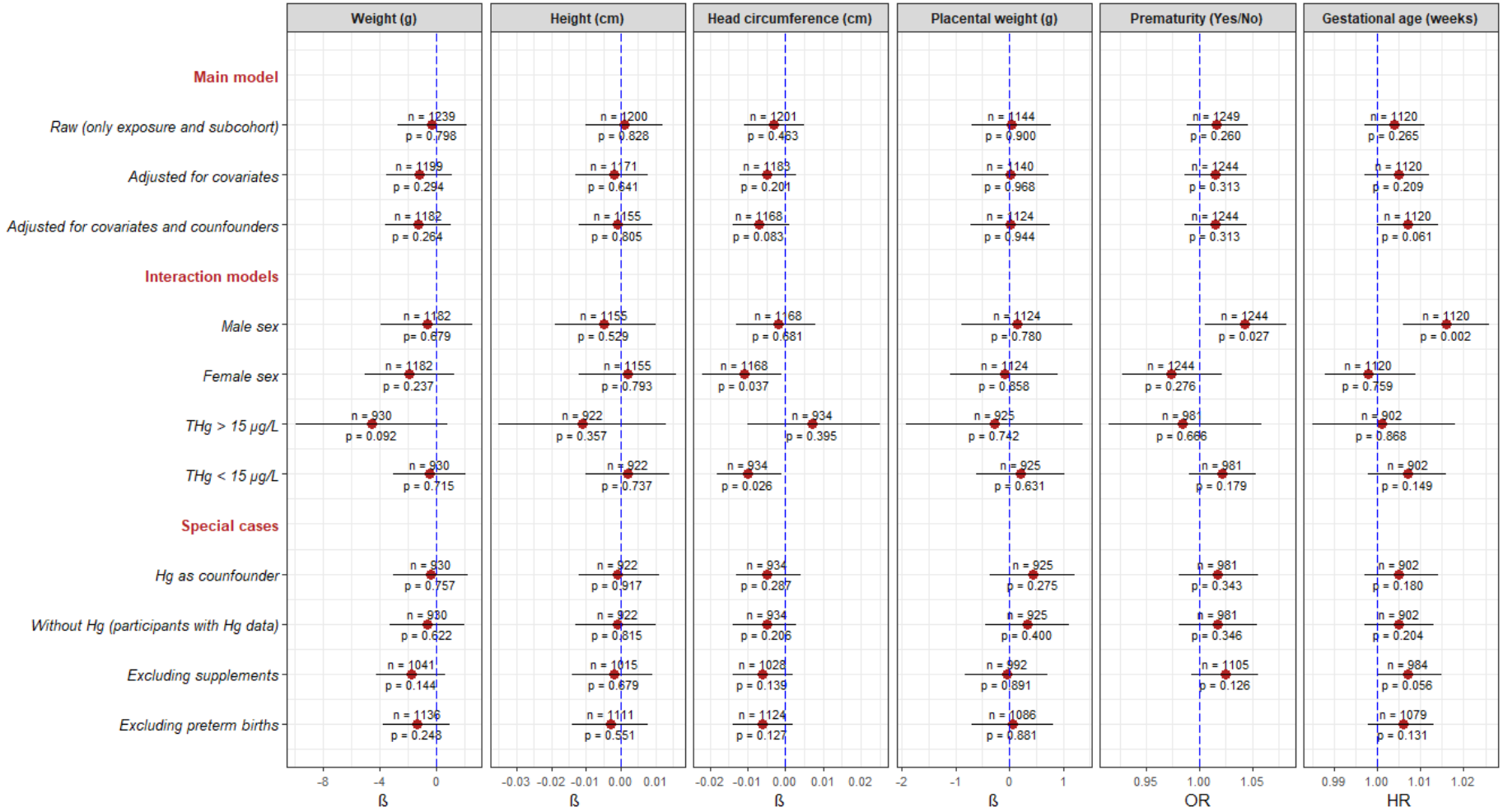
603**Figure 3.** Sensitivity analysis: raw model adjusted only for cohort, main model adjusted by covariates as Figure 1, main model adjusted by covariates and confounders, 604interaction analysis, and exclusion or inclusion of Hg, supplements consumption and preterm births. Adjusted association (95% CI) between maternal Se 605concentrations and outcome variables. INMA Project, 2004–2008 (Spain).



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607All models adjusted for cohort.

608Birth weight linear model additionally adjusted for covariates: parity, maternal smoking and exposure to smoking sources, maternal BMI, height, and country of birth at evaluation, child sex, paternal BMI and height; and additionally adjusted for confounders:
609maternal age and lean fish consumption.

Birth length linear model additionally adjusted for covariates: parity, maternal smoking and exposure to smoking sources, height, child sex and paternal height; and additionally adjusted for confounders: maternal age, country of birth, educational level and gestational age at evaluation.

Head circumference linear model additionally adjusted for covariates: parity, maternal smoking, height and educational level at evaluation, and child sex; and additionally adjusted for confounders: maternal age, country of birth, lean and oily fish consumption.

Placental weight linear model additionally adjusted for covariates: maternal BMI before pregnancy, parity, maternal working status at evaluation, parental social class, type of zone, maternal and paternal height; and additionally adjusted for confounders: maternal country of birth and educational level, lean and oily fish consumption.

Prematurity linear model additionally adjusted for covariates: maternal age, parity, maternal educational level. Without any significant confounders.

Gestational age COX model additionally adjusted for covariates: parity, type of zone, maternal height; and additionally adjusted for confounders: blood sampling season and lean fish consumption.

Table 1. Partial correlation matrix including outcome variables, Se and seafood intake variables.[a]

	-0.032

Abbreviations: BW, birth weight; BL, birth length; ; HC, head circumference; PW, placental weight; GL, gestational length.
[a]Seafood intake variables (servings per day).
[b]THg was log_2-transformed.
· p-value <0.10; * p-value <0.05; ** p-value <0.01; *** p-value <0.001.

623**Table 2**. Characteristics of mother-child pairs participating in the study. INMA Project, 2004–2006,
624Valencia and Gipuzkoa cohorts (Spain).

	N	%	Se (µg/L) Mean (SD)	ANOVA p-value
Cohort				
Gipuzkoa	1249	47.6	77.22 (10.61)	<0.001
Valencia		52.4	81.94 (7.92)	
Maternal variables				
Age (years)	1246	30.70 (4.08)[a]	0.055[b]	0.053[c]
Age (categorised)				
< 25 years	1246	6.3	77.71 (8.13)	0.170
25–29 years		32.2	79.40 (8.97)	
30–34 years		44.8	80.00 (9.46)	
≥ 35 years		16.7	80.25 (11.38)	
Country of birth				
Spain	1249	91.8	79.87 (9.70)	0.028
Other		8.2	77.70 (8.06)	
Education				
Up to primary	1247	22.9	79.58 (8.54)	0.106
Secondary		39.7	79.58 (8.95)	
University		37.4	79.84 (10.79)	
Social class				
I. II (highest)	1249	33.4	79.63 (10.32)	0.879
III		25	79.93 (9.92)	
IV. V (lowest)		41.6	79.60 (8.76)	
Employed during pregnancy				
No	1249	14.4	79.27 (8.82)	0.415
Yes		85.6	79.77 (9.71)	
Maternal height (cm)	1246	163.05 (6.14)[a]	-0.024[b]	0.401[c]
Pre-pregnancy BMI (kg/m^2)	1249	23.39 (4.19)[a]	0.014[b]	0.614[c]
Parity				
0	1249	54.4	79.69 (8.73)	0.973
1		38.4	79.72 (10.58)	
≥2		7.2	79.60 (10.32)	
Type of delivery				
Spontaneous	1182	79.0	79.46 (9.86)	0.015
Induced		17.6	81.59 (8.59)	
Caesarean		3.4	79.32 (8.85)	
Smoking at first trimester				
No	1230	81.3	79.77 (9.71)	0.888
Yes		18.7	79.87 (9.02)	
Exposure to sources of smoking				
No exposure	1223	34.1	79.68 (10.42)	0.688
1 exposure		40.7	79.53 (9.30)	
2 exposures		22.4	80.45 (8.86)	
≥3 exposures		2.8	78.85 (6.60)	
Area of residence				
Rural	1249	7.5	77.99 (11.65)	<0.001
Semi-urban		24.2	78.47 (8.79)	
Urban		68.3	80.31 (9.56)	

Season of blood sampling				
Spring	1249	26.3	80.43 (9.13)	<0.001
Summer		27.1	78.36 (8.72)	
Autumn		23.5	77.80 (9.67)	
Winter		23.1	82.35 (10.31)	
Gestational age at sampling	1249	13.08 (1.21)[a]	-0.199	<0.001[c]
Seafood consumption (sv/d)				
Lean fish	1232	0.19 (0.20)	0.098[b]	<0.001[c]
Oily fish		0.09 (0.10)	0.095[b]	<0.001[c]
Canned tuna		0.21 (0.21)	-0.014[b]	0.622[c]
Processed lean fish		0.05 (0.13)	0.070[b]	0.014[c]
Se supplementation (µg/d)	1249	1.88 (6.20)[a]	0.077[b]	0.006[c]
Se supplementation (categorised)				
Yes	1249	11.4	82.76 (7.86)	<0.001
No		88.6	79.30 (9.72)	
Paternal variables				
Education				
Up to primary	1241	35.5	80.32 (8.72)	0.214
Secondary		43.7	79.25 (9.93)	
University		20.8	79.53 (10.22)	
Paternal height (cm)	1242	176.30 (6.96)[a]	-0.021[b]	0.467[c]
Paternal BMI (kg/m^2)	1235	25.72 (3.35)[a]	0.059[b]	0.038[c]
Birth variables				
Sex				
Female	1249	48.1	79.50 (9.91)	0.490
Male		51.9	79.87 (9.29)	
Season of delivery				
Spring	1249	23.3	78.26 (9.83)	<0.001
Summer		22.4	81.77 (9.89)	
Autumn		27	80.90 (9.35)	
Winter		27.3	78.02 (9.35)	
Hg (µg/L) in cord blood	983	11.20 (8.91)[a]	0.231[b]	<0.001[c]
Categorised Hg in cord blood				
< 15 µg/L	983	75.5	78.95 (9.55)	<0.001
≥15 µg/L		24.5	82.81 (9.14)	
Outcome variables				
Gestational length (weeks)	1246	39.66 (1.56)[a]	-0.025[b]	0.384[c]
Preterm (<37 weeks of gestation)				
No	1249	95.8	79.61 (9.55)	0.156
Yes		4.2	81.52 (10.32)	
Anthropometry				
Birth weight (g)	1239	3342.67 (404.02)[a]	-0.020[b]	0.491[c]
Birth length (cm)	1200	49.91 (1.88)[a]	0.087[b]	0.002[c]
Birth head circumference (cm)	1201	34.51 (1.31)[a]	-0.072[b]	0.013[c]
Placental weight (g)	1144	609.82 (120.56)[a]	0.043[b]	0.144[c]

625[a] Mean (standard deviation)
626[b] Pearson correlation coefficient
627[c] p-value of Pearson's test

628**Table 3**. Multivariate linear regression analysis between maternal Se concentrations and
629anthropometric outcomes (n = 1249) with and without interaction with THg, sex, and season of
630blood collection.

	Main Se effect beta (95% CI), p	Se*THg p	Se*Sex p	Se*Season p
Birth weight (g)	-1.316 (-3.625 , 0.994), 0.264	0.164	0.590	0.766
Birth length (cm)	-0.001 (-0.012 , 0.009), 0.805	0.319	0.509	0.327
Head circumference (cm)	-0.007 (-0.014 , 0.001), 0.083•	0.075•	0.233	0.238
Placental weight (g)	0.026 (-0.712 , 0.764), 0.944	0.607	0.740	0.677
	Odds Ratio, p	**Se*THg p**	**Se*Sex p**	**Se*Season p**
Prematurity (yes/no)	1.015 (0.986 , 1.045), 0.312	0.767	0.025**	0.214
	Hazard Ratio, p	**Se*THg p**	**Se*Sex p**	**Se*Season p**
Gestational age (weeks)	1.007 (0.999 , 1.014), 0.061•	0.587	0.017**	0.827

631p: p-value.

632Values in parentheses are at 95% Confidence Interval

633THg: total mercury. Categorised as < 15 vs. ≥15 µg/L according to the equivalent for the WHO Provisional Tolerable Weekly Intake (1.6 µg/kg of body weight
634per week).

635All models adjusted for cohort.

636Birth weight linear model additionally adjusted for parity, maternal smoking and exposure to smoking sources, age, BMI, height, and country of birth at
637evaluation, child sex, paternal BMI and height, and lean fish consumption.

638Birth length linear model additionally adjusted for parity, maternal smoking and exposure to smoking sources, height, age, country of birth and educational
639level at evaluation, child sex and gestational age at evaluation, paternal height, blood sampling season, and lean, oily, canned tuna and processed lean fish
640consumption.

641Head circumference linear model additionally adjusted for parity, maternal smoking, height, age, country of birth and educational level at evaluation, child sex,
642lean and oily fish consumption.

643Placental weight linear model additionally adjusted for maternal BMI before pregnancy, parity, maternal working status at evaluation, parental social class,
644type of zone, maternal and paternal height, maternal country of birth and educational level, lean and oily fish consumption.

645Prematurity logistic linear model additionally adjusted for maternal age, parity, maternal educational level.

646Gestational age COX model additionally adjusted for parity, type of zone, maternal height, blood sampling season and lean fish consumption.

647• p-value <0.10; * p-value <0.05; ** p-value <0.01; *** p-value <0.001.

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